Effect of Aging on Chondrocyte Function

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Articular cartilage is a load-bearing tissue that provides a frictionless and wear-resistant surface during joint movement. The cartilage can maintain this function for many decades in healthy joints; however, under the pathological conditions of osteoarthritis (OA), degeneration of cartilage leads to loss of mechanical integrity and motion (Fig. 1). In aging, articular cartilage stands out as a unique tissue since the cells (chondrocytes) and the majority of the extracellular matrix proteins (proteoglycans, collagen, and noncollagenous proteins) experience little turnover, resulting in a tissue that must withstand the accumulation of years of aging-associated changes without significant repair or renewal.

We have hypothesized that the age-dependent decreases in cell synthetic activity are due to a reduction in cell-matrix interactions that occur as a function of age. We have proposed to examine mechanisms by which aged cells become less responsive to mechanical loading, simulating the normal activity of aging human joints. If successful, the findings of this proposal will support the notion that healthy aging results in a loss of binding adhesion of aged cells to the matrix, which results in an understimulated cell synthesis and thus loss of homeostasis.

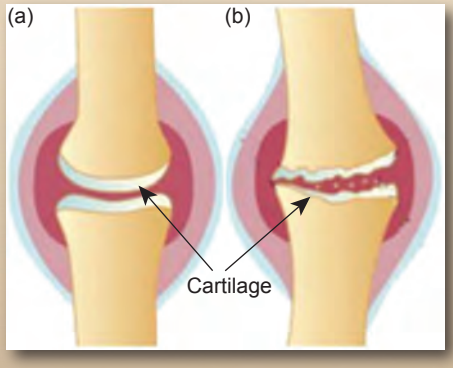

Figure 1. Cartilage in (a) healthy individuals, and (b) individuals with OA.

Project Goals

The project uses molecular biology (isolating live cells from bovine joints), biochemistry (purifying and modifying extra cellular matrix proteins from bovine joints to examine cell-protein adhesion), and bioengineering (single cell measurements using atomic force microscopy (AFM)) tools to examine age-dependent changes in molecular and mechanical properties of chondrocytes.

Three major outcomes are anticipated from these experiments. First, we aim to determine Young's modulus (compressive stiffness) and Poisson's ratio (resistance to lateral expansion) as a way to characterize physical properties of immature, young, and aged chondrocytes. We anticipate that age-dependent variations will be observed. Second, we aim to determine the molecular binding adhesion values of young and aged chondrocytes to extracellular matrix proteins that are also isolated from young or old joints. We anticipate that age-dependent changes in adhesion forces will be observed. Third, we aim to develop methods for purifying chondrocytes from mouse cells and compare them to bovine cells (the standard cells used in the field).

The long-term goal is to develop novel approaches of *in vitro* and *in vivo* analysis that can be carried out in an animal system amenable to genetic manipulations. If successful, the potential findings of this proposal could help establish a paradigm shift in the current dogma of age-dependent loss of chondrocyte function. The findings will also offer new insights into potential new target matrix proteins and receptors responsible for the decreased sensitivity of aged chondrocytes to loading. Such targets may represent novel venues for the development of alternative pharmaceutical treatments of OA.

Relevance to LLNL Mission

With the increase in our aging population, instances of OA will eventually reach epidemic proportions, creating a great economic burden on our healthcare system. In addition, excessive use and injuries to joints, commonly associated with intense physical training of our armed troops, can potentially increase the risk of developing early onset OA in young cadets, and chronically affecting the elderly veteran population. Understanding the age-related changes in biomechanical stimulation and chondrocyte function is vital for the development of OA treatments.

Our work fits into the measurement goals of LLNL's Science and Technology Pillars. Our research aim is to develop measurements for single cells using AFM, which can have applications in biomedical countermeasures.

FY2009 Accomplishments and Results

Although many approaches have been developed to model the deformation of chondrocytes *in situ*, existing theoretical models have not been able to sufficiently account for the empirical *in situ* magnitude of strain that cells are exposed to during cyclic loading. This limitation may be due to the assumption

that cells exhibit a constant compressive stiffness, despite their heterogeneous structure. We hypothesized that chondrocytes exhibit a nonlinear elastic modulus, requiring a non-Hertzian analysis of the contact of the resulting stress-strain response. To examine the indentation-dependent stiffness of chondrocytes using an AFM, the response of chondrocytes was investigated by probing the bulk stiffness of the cell using 10-µm spherical probes under different dynamical loading rates and relaxation times. The data was fit to an indentation-dependent model to empirically calculate the nonlinear elastic modulus as a function of deformation (Fig. 2).

We found that the mechanical stiffness of chondrocytes is dependent on the time the load is applied (hold time) and the time between subsequent loads (relax time) at a rapid loading rate (10 mm/s). We also found that the mechanical stiffness is not dependent on the hold time or relaxation time at a slow loading rate (1 mm/s). The dependency of elastic modulus on the hold and relaxation time at rapid loading rates suggests that the cell is deformed into a nonequilibrium conformation and the cells require anywhere from 1 to 10 s to equilibrate to a normal conformation. We believe this nonequilibrium configuration at rapid loading rates originates from the dynamic fluid rearrangement that occurs within the cell.

Related References

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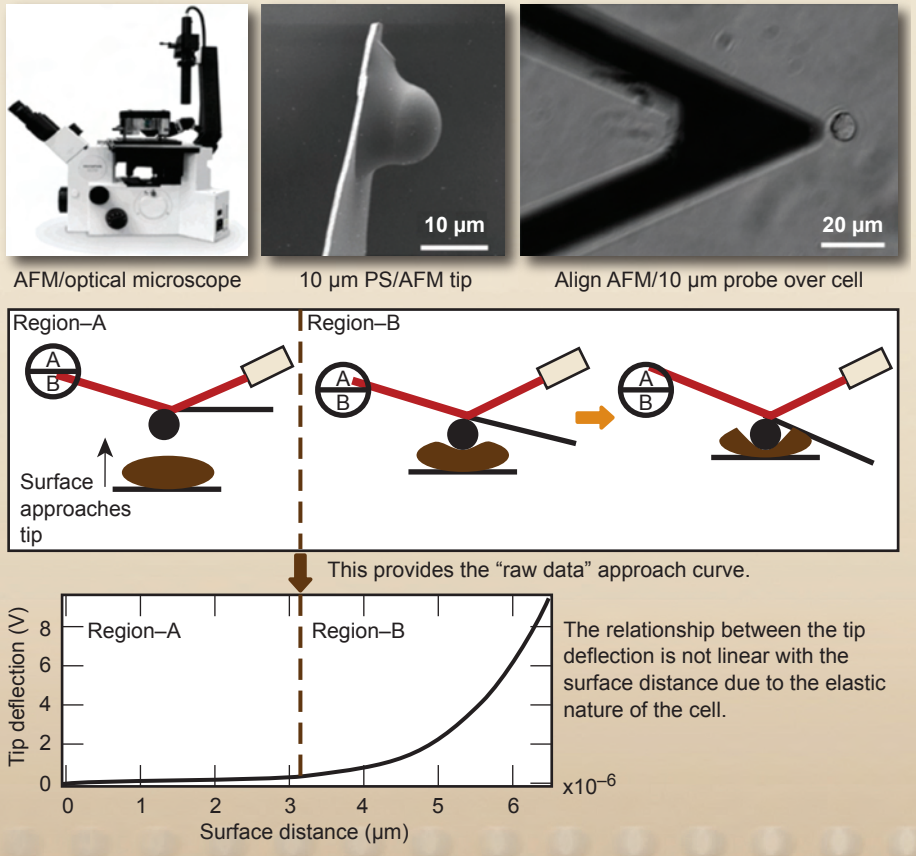

Figure 2. Process to measure the mechanical response of a cell to loading. Indentation curves were acquired where the AFM cantilever was initially not in contact with the surface (Region A). As the surface contacted the cell, the cantilever was deflected upwards, which was measured on the position sensitive detector (Region B). The nonlinear response of the cell surface to the applied force was then used to calculate the indentation-dependent stiffness of the cells.

FY2010 Proposed Work

In FY2010 we will focus on examining age-dependent changes in binding adhesion of cells and matrix proteins. We will conjugate two bovine proteins (hyaluronan and collagen type II) to beads and glass substrates and measure the adhesion forces between immature or adult chondrocytes and proteins attached to beads. We will also obtain mechanical measurements to determine if young and old chondrocytes have different affinities for extracellular proteins derived from young and old joints. In parallel experiments, we will isolate RNA from chondrocytes labeled with green fluorescent protein and carry out microarray experiments.